THE ORIGIN OF THE CHLOROPLASTS IN THE COTYLEDONS OF HELIANTHUS ANNUUS

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(WITH PLATE XXI)

There are two prevalent opinions in regard to the origin of chloroplasts in seedlings. One group of investigators holds the view that the chloroplasts originate directly from the cytoplasm of the cell. According to them, the mature seed from which the seedling originates contains no chloroplasts. They hold that if any chloroplasts are present in the young embryo, they lose their color and disintegrate at the ripening of the seed, and that at germination the protoplasm in the cells of the cotyledons gives rise to new chloroplasts which function during the period of activity of these organs. The other group of investigators maintains that the protoplasm of the cell never gives rise to chloroplasts, but that the fertilized egg contains chromatophores which have been derived directly from the parent plant. During the development of the egg into the embryo these chromatophores multiply, and in this manner provide every cell of the embryo with chromatophores. In many seeds before maturity the chromatophores have become differentiated into chloroplasts, which are plainly visible. During the ripening of these seeds their chloroplasts lose their color and shrivel up, and on this account they are difficult to detect in the mature seed. At germination, however, the chloroplasts again become active and assume their original form and color.

SACHS (1) states that the chloroplasts arise in the young cells by the separation of the protoplasm into portions which remain colorless and others which become green and sharply defined. He holds that the process can take place by very small particles, originally of a different nature from the apparently homogeneous protoplasm in which they are distributed, collecting at definite places and appearing as separate masses.

MIKOSCH (**2**), after an examination of the seeds and seedlings of *Helianthus annuus*, reached the conclusion that there are no chromatophores present in the resting seeds of this plant, but that during germination the chloroplasts arise directly from the protoplasm of the cells. He holds that their origin is due to the condensation of the cell plasma in definite places. This condensation he thinks is probably brought about by a loss of water in those parts. The condensed parts soon take on the green color and become the chloroplasts. This process of formation goes on independently of light. The bodies thus formed are at first rod or spindle-shaped, but later assume the typical disk shape of the choloroplasts.

BELZUNG (**3**), after a lengthy investigation of the ripening seeds as well as of the mature seeds and seedlings of many plants, came to the following conclusions: (1) that the free growth of starch grains can take place without the intervention of leucoplasts; (2) that the chloroplasts are formed directly by a differentiation of the protoplasm; (3) that the chloroplasts can also be formed at the expense of the starch grains which have their origin in the cytoplasm of the cell. The severe criticism of his work by SCHIMPER (**4**) led BELZUNG to traverse anew his previous work, and in a paper published in 1891 (**5**) he verified his previous conclusions. In this investigation he used as material *Phaseolus vulgaris*, *Lupinus albus*, *Lupinus elegans*, *Faba vulgaris*, *Pisum sativum*, and other plants. According to his observations, the young embryo contains no chloroplasts. The starch grains formed in the young embryo are laid down in the vacuoles of the protoplasm. He holds that those who claim that starch grains are the product of leucoplasts are in error, and that the leucoplasts defined by different investigators are simply the boundaries of the vacuoles in the protoplasm. The green color of the embryo in many plants is due to a green pigment distributed throughout the protoplasm of the cell. According to BELZUNG, therefore, there are no chromatophores present in the embryo, and consequently none in the mature seeds. At germination the simple starch grains of the seed disintegrate, and numerous compound grains of transitory starch appear in various parts of the protoplasm. These com-

pound grains are formed as follows. Each large vacuole is composed of two to five secondary ones. In each of the latter a small starch grain is formed, the several grains making the compound grain. As this grain disappears, an infiltration of the green pigment takes place and thus a chloroplast is formed. BELZUNG made most of his observations upon fresh material and used iodine green for his staining.

MEYER (**6**), after his thorough investigation of the structure and nature of the chloroplasts, reached the conclusion that the origin of the chromatophores does not take place in the young plant cells, but that they are derived from other cells in which they previously existed, and that they increase in number by the division of those already present.

SCHIMPER (**7**) found chromatophores present in the embryo sac and egg of numerous phanerogams. Although his observations were rather meager, he concluded that the chloroplasts thus present in the young embryo were not reabsorbed in the ripening seed, but that they merely become colorless and lose their function. Upon germination, after they have again taken on the green color, they become functional.

BREDOW (**8**) examined a large number of green, yellowish, and colorless seeds, and came to the conclusion that chloroplasts were present in all of them, although they stain very poorly and are hard to detect. He studied the seeds of *Pisum sativum*, *Robinia Pseudacacia*, *Cucurbita Pepo*, *Acer crataegifolium*, *Ipomoea splendens*, *Pinus austriaca*, and *Lupinus luteus*, both in the fresh condition and after the treatment with reagents. The sections of the fresh seeds were mounted in cell sap or in weak glycerin. Good results in staining the chloroplasts were obtained by treating the sections for several days with a concentrated solution of picric acid. This colored all the proteid material yellow, but the chloroplasts showed a deeper stain than the other cell contents. In sections treated with picric acid, washed with water, and then stained with hematoxylin, the chloroplasts also showed well. BREDOW found that the chloroplasts of the seed increased during germination by simple fission, and also by a division of one chloroplast into as many as ten or twelve smaller ones by numerous irregular

divisions. He believed that the greening of these numerous small bodies led the earlier investigators to the conclusion that the chloroplast originates directly from the protoplasm of the cells of the seedling. It is worthy of note that BREDOW worked with *Lupinus luteus*, a seed in which BELZUNG found no chloroplasts at all.

FAMINTZIN (9) investigated the origin of chloroplasts in seedlings, and especially the manner in which the chloroplasts, if present in the seed, divide. He selected as his material for investigation the seeds and seedlings of the sunflower, using the fresh material of seeds and of 16 and 24-hour seedlings. By ZIMMERMAN'S method he was unable to distinguish the small aleurone grains and particles of proteid from the chloroplasts, since the whole cell content stained red. He then originated a modification of this method by treating the sections previous to fixing and staining with acetic acid. The sections were left in 1 per cent acetic acid for 24 hours, or less for a stronger solution of the acid, and were then fixed and stained according to the method of ZIMMERMAN. By this means the protein granules and grains remained colorless or were only faintly colored red, while the chloroplasts and other protoplasmic structures were stained a deep red. In this way FAMINTZIN was able to make out the chloroplasts in the resting seed and during the early stages of the germination. Some of the chloroplasts in the resting seed are in the cytoplasm lining the cell wall, but by far the greater part of them, according to him, are in the film of protoplasm which surrounds the protein grains. Upon placing fresh sections of the material in the light, he observed that these small bodies on the protein grains took on a yellowish or brownish color. By identifying these bodies in the young stages of the seedlings, FAMINTZIN concluded that the seeds of the sunflower contain chloroplasts, and that these by simple fission give rise to those of the seedling.

The seeds for the following investigation were planted in white quartz sand and placed in the greenhouse at a temperature of 65–75° F. At intervals of 12 hours the seedlings were taken up, and parts of the cotyledons near the middle were placed in the fixing material. This was carried on until the plants were above

the ground and had become true photosynthetic structures. The seedlings were examined at 11 different stages exclusive of the seed. Chromacetic acid solution was used for fixing, and the material was washed, dehydrated, and imbedded in paraffin in the usual manner. Various methods of staining were tried. By the use of ZIMMERMAN'S method the same difficulties were encountered as were experienced by FAMINTZIN. In the later stages of the seedlings the chloroplasts are plainly differentiated, but during the early stages they could not be distinguished at all from the protein granules in the cell. Sections which had been treated with picric acid and then tinged with eosin also showed the chloroplasts plainly in the later stages of the seedling, but during the earlier stages the protein matter, as well as the chloroplasts, takes a deep stain and the identity of the latter is uncertain. Sections were then treated according to FAMINTZIN'S modification of ZIMMERMAN'S method. One series of sections was placed in 30 per cent acetic acid for 30 minutes, then washed with running water and transferred to 0.2 per cent acid fuchsin. After being allowed to stand for 24 hours in this solution, the sections were washed in running water for 12 hours, dehydrated in 95 per cent and absolute alcohol, cleared in xylol, and mounted in balsam. Another series of sections was treated in the same manner, except that they were left in the 30 per cent acetic acid 45 minutes. The results obtained from the last series were the most satisfactory, and the examination of the material was made upon these sections.

The sections first examined were those of seedlings which were fully developed, and the number and position of the chloroplasts in the cell were clearly evident (fig. 12). Those next examined were of seedlings 12 hours younger, and fig. 11 shows the usual position of the chloroplasts at this stage. This method was continued step by step back to the original seed, since obviously the best means to find the nature of the origin of the chloroplasts is to trace them backward in this manner from stages in which there can be no doubt at all as to their position and identity. The chloroplasts, as shown clearly in figs. 1–12, occupy the normal position in the cytoplasm of the cell at all stages of the development of the seedling. In the resting seed, according to our opinion,

they are present in their usual place but are very minute. As the seed begins to germinate, they increase in size and then begin to divide by simple fission. The chloroplasts of the seed are thus the bodies which give rise to the chloroplasts of the mature seedling. The numerous small round bodies which are on the surface of the protein grains, and which take the red stain after the same manner as the chloroplasts, we do not consider as chloroplasts, since in the first place they are entirely too numerous to correspond with the number of chloroplasts in cells where their identity cannot be doubted, while the number of chloroplasts found in their normal position closely corresponds to the number which is found in the later stages of development. Also, these small bodies are plainly evident upon the protein granules in advanced stages of germination, when there is not the least doubt as to the identity of the chloroplasts. We agree with FAMINTZIN that the chloroplasts are present in the resting seeds of *Helianthus annuus*, and that they alone give rise to those of the seedling. We think, however, that he is in error in considering the small bodies which cover the protein granules as chloroplasts. What these bodies are we are unable to tell, but it seems evident that the stained bodies observed in the natural position of the chloroplasts account for all which appear in the seedling.

In conclusion I desire to extend my sincere thanks to Professor A. W. EVANS, at whose suggestion the work was undertaken, for his able advice and criticism; also to Dr. GEORGE E. NICHOLS, who gave me valuable advice in the preparation of material.

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EXPLANATION OF PLATE XXI

The abbreviations used are as follows: *c*, chloroplast; *t*, protoplasm; *p*, protein grains; *pf*, protein grain with bodies designated as chloroplasts by FAMINTZIN; *dp*, protein grains beginning to disintegrate; *n*, nucleus; *pt*, protoplasm and disintegrated protein grains; *v*, vacuole.

FIG. 1.—A lower palisade cell from a cotyledon of the resting seed; the protein reserve is seen in the form of large grains (*p*); the chloroplasts are seen in their usual position near to the cell wall; seven chloroplasts are visible in this section; ×600.

FIG. 2.—A palisade cell from the cotyledon of a seed 12 hours after planting; the protein grains are still intact; the protoplasm has become vacuolated and active; at either end of the section of the cell the chloroplasts are seen; ×600.

FIG. 3.—A palisade cell from the cotyledon of a seed 24 hours after planting; the protoplasm has become dense and shows no definite structure; the chloroplasts are still small; ×600.

FIG. 4.—A cell from the palisade layer of the cotyledon of the seed 36 hours after planting; the chloroplasts (*c*) have increased in size; the rudimentary hypocotyl and root of the seed have not yet penetrated the seed coats; ×600.

FIG. 5.—A palisade cell 48 hours after the planting of the seed; on the protein granules (*pf*) may be seen the bodies designated by FAMINTZIN as chloroplasts; the hypocotyls in the seedlings have a length of 0.6 cm.; ×600.

FIG. 6.—A cell from the spongy layer of a cotyledon in a 60-hour seedling; the protein granules (*dp*) are plainly disintegrating; with the stain used, the nucleus first shows at this stage; ×600.

FIG. 7.—A cell from the spongy layer of a cotyledon of a 72-hour seedling; the hypocotyls of the seedlings have reached a length of 2.5–3.5 cm.; the protein grains have now disintegrated and the cell content has become very dense; the chloroplasts are beginning to increase by division; ×600.

FIGS. 8–11.—Cells from 84, 96, 108, and 120-hour seedlings; the increase in size and number of the chloroplasts at different stages can be plainly seen; during each succeeding stage the cell content becomes less dense and vacuoles appear; ×600.

FIG. 12.—A palisade cell from a cotyledon of a 140-hour seedling; the seedling has become independent and the cotyledon is a typical foliage organ; ×600.